onic lethal mutation that was identified based on the near absence of the expression of *crestin* by trunk neural crest cells in mutant embryos. *hbk* mutant embryos completely lack chromatophores, as well as sympathetic, dorsal root ganglion, and enteric neurons. The absence of these derivatives is preceded by abnormal neural crest expression of multiple transcription factors known to regulate early neural crest development. *hbk* mutant embryos also display defects in cardiovascular development as evidenced by cardiac edema and a lack of blood and circulation. Abnormal expression of several genes known to regulate the development of cardiac, vascular, and hematopoietic progenitors has also been observed. Neural plate and axial and paraxial mesoderm development in *hbk* mutants is indistinguishable from wild-type siblings. These observations suggest *hbk* function is specifically required for neural crest induction and/or early development, as well as for the establishment and development of specific lateral plate mesoderm-derived lineages.

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Lineage analysis of Rohon–Beard sensory neurons and neural crest cells

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Neural crest cells (NCCs) and Rohon–Beard sensory neurons (RBs) both arise from the same region in developing embryos. NCCs migrate extensively and ultimately give rise to several cell types, including most of the peripheral nervous system. RBs are mechanosensory neurons whose cell bodies reside in the central nervous system of anamniote vertebrate embryos. Analysis of several zebrafish mutations suggests that these cell types may share a common progenitor, but it remains to be determined when and if this is the case. I hypothesize that a population of precursor cells exists prior to differentiation of cells at the neural plate border that is capable of giving rise to both NCCs and RB neurons, and that some of these precursors will ultimately give rise to both cell types. If the hypothesis is supported, a link will be established between cells in the central nervous system and the neural crest. The current project addresses this hypothesis using the lipophilic dye DiI to label regions in the developing zebrafish embryo and map precursor cells for RBs and NCCS. Embryos are labeled in the stages prior to and including 90% epiboly, at which time the progenitors are thought to reside at the border of the neural plate and non-neural ectoderm, and are later examined for the presence of labeled RBs and/or NCCs. Preliminary data suggest the trunk neural crest precursors reside in the ventral posterior region of the embryo, and RB neurons potentially come from this same region.

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Epithelial–mesenchymal transition regulators Snail and Twist are required for PMC ingression in the sea urchin embryo

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Epithelial–mesenchymal transitions (EMTs) are fundamental to embryonic morphogenesis throughout the animal kingdom. At the onset of gastrulation in the sea urchin embryo, micromere-derived primary mesenchyme cells (PMCs) undergo an EMT process to ingress into the blastocele, and these cells later become the larval skeleton. Transcriptional regulators such as Snail and Twist have emerged as important molecules for controlling EMTs in many systems. Sea urchin *snail* and *twist* genes were cloned and characterized from *Lytechinus variegates*. As expected, functional knockdown analyses of Snail with morpholino-substituted antisense oligonucleotides in whole embryos and chimeras demonstrated that Snail is required in micromeres for PMC ingression. We proceeded to place Snail in the sea urchin micromere–PMC gene regulatory network (GRN) as a key regulator of PMC ingression. Snail is expressed late in micromere specification downstream of Alx1 and is essential for ingression. Phenotypes observed in Twist-deficient embryos indicate that Twist is also required for PMC ingression. Current efforts are to place Twist in the micromere network. Taken together, these data place Snail and Twist in the context of the PMC specification program and show these transcription factors to occupy central positions in the subnetwork that regulates EMTs in the sea urchin embryo.

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Clearing the way: The small GTPase RhoA and endomesodermal specification

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The monomeric GTP-binding protein RhoA is best known for its role in morphogenesis and cytoskeletal remodeling. Further research identifies RhoA involvement in cell polarity, cytokinesis, and dorsal closure. Studies in the sea urchin *L. variegatus* suggest a novel role for RhoA in endomesodermal specification. Near the top of the network specifying endomesoderm in the sea urchin is Wnt8, which positively regulates beta-catenin signaling in a community effect that drives the entire network. We have discovered that RhoA works in the Wnt8 pathway and identified a role for RhoA in endomesodermal SoxB1 clearance. For endomesodermal cells to be fully specified, SoxB1 must be progressively cleared from the nuclei of vegetal cells, where SoxB1 is thought to interfere with beta-catenin signaling. Our data reveals that when co-expressed with dominant negative Wnt8, activated RhoA is capable of rescuing SoxB1clearance (as well as the expression of the downstream endodermal

specifier GataE and the endoderm marker Endo16). These results demonstrate that RhoA is indeed involved in specification events and suggest that embryonic RhoA activity is even more complex than formerly recognized. Among our current studies, we aim to determine whether RhoA is upstream of SoxB1 transcription and/or protein degradation.

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The *Ciona intestinalis* MyoD homolog is essential for myogenesis

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We used gene over expression and gene knockdown to study the functional properties of CiMDF, the single myogenic regulatory factor gene (MRF) of *Ciona intestinalis*. Overexpression was achieved by injecting eggs with synthetic *CiMDF* mRNA. RNA-injected eggs did not develop typical tadpole morphology, but instead formed flattened balls of cells. Expression of the four muscle-specific genes assayed was strongly upregulated in muscle cells of injected embryos. Notably, these muscle-specific genes were also expressed in non-muscle lineage cells of injected embryos. This was especially apparent for *troponin* I, which was typically detected in all vegetal hemisphere cells of mRNA-injected embryos. Animal hemisphere cells of mRNA-injected embryos did not express muscle genes. We knocked down CiMDF expression by injecting eggs with antisense morpholino oligonucleotides (MO). MO-injected eggs often developed into quite normal tadpoles with clearly recognizable heads and tails; nevertheless, muscle-specific gene expression was reduced in these embryos compared to controls. Moreover, MO-treated larvae were almost always paralyzed and never showed the frenzied tail movements of normal larvae. Electron microscopy revealed that "muscle" cells of MO-treated larvae lacked the myofibrils seen in normal larval muscle. We conclude that *CiMDF* is a bona fide MRF because it elicits ectopic myogenesis, and that it is essential for tail muscle development. Awards from the NIH (1 R15 HD047357-01) and Rhode Island College to THM, and the Association Francaise contre les Myopathies (AFM) to HY supported this work.

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TGF-beta and LIN-12/Notch signaling pathways regulate dorsal–ventral patterning of the *C. elegans* post-embryonic mesoderm

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A small number of signaling pathways including TGF-beta and Notch are used repeatedly in many different developmental contexts. While the core components of these pathways have been well studied, the mechanisms involved in regulating the specific signaling output in different cell types are less well understood. We are studying mesodermal patterning and cell fate specification in *C. elegans*. We have determined that SMA-9, the homolog of the *Drosophila* protein Schnurri, is required for the dorsal–ventral asymmetry of the *C. elegans* post-embryonic mesoderm, the M lineage. Animals with mutations in *sma-9* exhibit a dorsal to ventral fate transformation within the M lineage. Through a *sma-9* suppressor screen, we have uncovered a role for the Sma/Mab TGF-beta signaling pathway in the M lineage. We show that SMA-9 specifically antagonizes Sma/Mab TGF-beta signaling in the M lineage, implicating a novel mode of function for the SMA-9/ Schnurri family of proteins. We also investigated the role of the LIN-12/Notch signaling pathway in regulating dorsal–ventral patterning in the M lineage. Our results are consistent with a model that the Notch and TGF-beta signaling pathways function asymmetrically to regulate dorsal–ventral patterning and that SMA-9 functions to antagonize TGF-beta signaling in dorsally fated cells. Our findings provide support for an evolutionarily conserved function of SMA-9/Schnurri in regulating TGF-beta signaling in dorsal–ventral patterning.

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FACS-assisted microdissection of photolabeled cells to identify germ layer-specific genes

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We have developed a method for rapidly identifying regionally expressed molecules in multicellular organisms: FACS-assisted microdissection of photolabeled cells (FAM-P). For our first FAM-P project, we injected zebrafish embryos with purified Kaede protein, a stony coral protein that fluoresces at 514nm (green) prior to – and 582nm (red) after – photoconversion at 405nm. We used the scanning laser of a confocal microscope to label twelve late blastula stage embryos along the 4–6 tiers of blastula margin cells, comprising the mesoderm and endoderm germ layer precursors. Labeled embryonic cells were dissociated and subjected to fluorescence-activated cell sorting (FACS), separating red mesoderm/endoderm precursors from green ectoderm precursors. RNA was extracted from these separated cells, linearly amplified and labeled, and then hybridized to a microarray with over 30,000 oligonucleotides representing more than 20,000 unique zebrafish genes. In validation of our strategy, a statistically significant number of genes with known elevated expression in the late blastula margin were re-identified by this approach. We also identified dozens of genes enriched in the mesoderm/endoderm